Original Article

Comparative Analysis of PTEN, ER, and PR Expression in Invasive Versus Non-Invasive Breast Carcinoma Across Different Histological Grades in Local Population

Zunaira Qayyum[1], Sadaf Zahid[2], Shagufta Jabeen Mirza[3], Sakina Jamil[4], Ayesha Mobeen[5], Arfa Naeem[6]

[1]Assistant Professor Department of Pathology MBBS-MC Mirpur Azad Kashmir
[2] Department of Pathology, SIMS college Lahore
[3]Demonstrator Department of Pathology MBBS-MC Mirpur Azad Kashmir
[4]Assistant Professor of Pathology Pak Red Crescent Medical and Dental College, Lahore
[5]Post Graduate Resident Histopathology Department of Histopathology Sheikh Zayed Hospital Lahore
[6]Assistant professor of Pathology Central Park Medical College, Lahore

Correspondence: *Dr. Zunaira Qayyum*
Assistant Professor Department of Pathology MBBS-MC Mirpur Azad Kashmir
zuniqayyum@gmail.com

Abstract

Objective: The purposes of this study to compare PTEN, ER, and PR expressions between invasive and non-invasive breast carcinoma; and to analyse their expressions according to histological grades.

Methodology: This retrospective cohort study was conducted department of pathology MBBS-MC Mirpur, AJK from January 2013 and December 2023. The study consisted of sample of two hundred female patients distributed into two groups, invasive breast carcinoma with one hundred patients and non-invasive breast carcinoma with the other one hundred patients. Histological grading was done according to the Nottingham Grading System guidelines. To measure the stain intensity and the proportion of positively stained cells in relation to PTEN, ER, and PR, immunohistochemistry encircled by antibodies was employed.

Result: The mean H-score of PTEN expression in invasive carcinoma was 4.2 ± 1.7. Majority of patients in this study were staged at T2NOMO while the distribution of tumour stage in non-invasive carcinoma was T1 (n= 6), T2 (n= 11), and T3 (n=39). On average, patients with PFO reported to have had 3 ± 1.4 episodes. For ER, the mean H-score was 5 and tumour invasiveness was found associated with a higher H-score. The mean H-score was found 8±2.1 in invasive tumors and in non-invasive tumors, it was 7. As for PR expression, the mean H-score was 5. Based on histological grading it was noted that well differentiated or Grade 1 tumor sample had the highest scores of the biomarkers where PTEN stood at 6, and ER at 7. The poorly differentiated (Grade 3) tumors expressed the lowest levels of PTEN at 3.2 ±1.8, ER at 4.5±2.2, and PR at 4.0±2.0 for the solution. Spearman's rho test revealed statistically significant moderate positive correlation between ER and PR (rho = 0. 64, p < 0. 001) while PTEN has statistically significant weak negative correlation with both ER (rho = - 0. 32, p = 0. 02) and PR (rho = - 0.28, p = 0. 03).

Conclusion: There was a significant difference in the PTEN, ER, and PR expression in invasive and non-invasive breast carcinoma as well as in different histological grades. PTEN, ER and PR were higher in non-invasive carcinomas than the invasive carcinomas and in well differentiated than in poorly differentiated carcinomas.

Keywords: PTEN, Estrogen Receptor (ER), Progesterone Receptor (PR), invasive breast carcinoma, non-invasive breast carcinoma, histological grading, immunohistochemistry, biomarkers.

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Introduction

Breast carcinoma is one of the most common diseases with a great variability of the clinical manifestation that is characterized by the tumor proliferation of epithelial tissue of the ducts or lobules of the breast. It is the predominant malignancy affecting female subjects globally and one of the main causes of cancer mortalities;[1] It is for this reason that breast cancer has been classified as being heterogeneous and the search for biomarkers to use in diagnosing, assessing prognosis and in the treatment is necessary. Biomarkers are needed since they can help to understand the molecular characteristics of cancer development and help to identify patients for the personalized treatment. Out of all these biomarkers, PTEN (Phosphatase and Tensin Homolog), ER (Estrogen Receptor), and PR (Progesterone Receptor) have especially received much attention because of their involvement with tumorigenesis as well as their relation to

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therapeutic treatment. [2,3]

PTEN a tumor suppressor gene that plays a role in cell proliferation, survival and migration. The down regulation, or loss of this gene is well linked to cancer progression and increment in the mortality rate.[4] Estrogen Receptor (ER) and Progesterone Receptor (PR) are considering members of nuclear hormone receptors that have key functions in the biology of breast cancer. ER-positive breast cancers usually are more sensitive to hormonal manipulation hence making ER status as one of the indicators for managing treatment. PR is estrogen responsive and reflects functional estrogen signaling. The existence of PR is related to higher degrees of differentiation and a superior prognosis. [5,6]

Thus, PTEN, ER, and PR are important biomarkers giving rather accurate data on molecular type of BC and helping to design adequate therapeutic management. Such comparison of invasive and non-invasive breast carcinoma is highly significant in solving the problems related to the analysis of carcinoma progression and searching for new therapeutic targets. Breast carcinoma that has penetrated the basement membrane and involves surrounding tissues is invasive, while cancer cells that have not crossed the basement membrane and remain within ducts or lobules is non-invasive presenting a lesser clinical aliveness.[7,8]

It can be expected that invasive carcinomas can have even a less expression of PTEN and the change of ER and PR comparing with non-invasive carcinomas. The dysfunction of the PTEN is linked with aggressiveness of the tumor coupled with hormonal receptor status.[9] Patients with higher histological grade, which implies poorly differentiated and more aggressive tumors, could have the lower PTEN levels and/or different hormonal receptor status in comparison with patients with lower tumor markers. The changes in molecular basis of tumor progression are triggered by corresponding changes in the histological picture of the tumor. Knowledge of such relations will disclose beneficial prognostic evidence and may reveal novel therapeutic or preventative targets.[10]

This present study has been planned to investigate PTEN, ER, and PR staining patterns in invasive-and non-invasive breast carcinoma in the local population based on the tumour's histological grade. Which will provide a better understanding of various spikes and interactions of breast cancer biomarkers and their significance to prognosis or treatment in our local population.

Methodology

This retrospective cohort study was conducted in the department of pathology, MBBS-MC Mirpur AJK after taking ethical approval form hospital ethical committee. The study used retrospective data of breast cancer surgeries which were done between January 2013 and December 2023.

Patients included in this study were selected based on specific inclusion and exclusion criteria. Inclusion criteria consisted of female patients aged 18 years or older who had been diagnosed with histologically confirmed invasive or non-invasive breast carcinoma and had undergone biopsy or surgical excision. Patients with recurrent breast cancer, those who had received neoadjuvant therapy prior to sample collection, or cases with insufficient tissue samples for immunohistochemical analysis were excluded to avoid confounding factors that could affect biomarker expression.

The sampling process began with the retrieval of formalin-fixed, paraffin-embedded (FFPE) tissue blocks from the pathology archives. These tissue blocks originated from core needle biopsies, lumpectomies, or mastectomies, representing a comprehensive range of breast cancer specimens. For each selected case, detailed demographic and clinical information, including age at diagnosis, tumor size, lymph node status, and histological type, were meticulously recorded. The final study cohort consisted of 200 cases, evenly divided between invasive (100 cases) and non-invasive (100 cases) breast carcinoma. This sample size was determined to be adequate for detecting statistically significant differences in biomarker expression, assuming a 95% confidence level and 80% power.

Histological grading of breast carcinoma was performed using the modified Bloom-Richardson grading system. Tumors were classified into three grades: Grade 1 (well-differentiated), Grade 2 (moderately differentiated), and Grade 3 (poorly differentiated).[11] The grading process was conducted independently by two experienced pathologists to ensure reliability and minimize inter-observer variability. In cases where discrepancies arose, the pathologists reached a consensus through joint review and discussion of the histological features.

Immunohistochemical staining was carried out on 4-µm-thick sections cut from the FFPE tissue blocks. The sections underwent deparaffinization in xylene, followed by rehydration through a series of graded alcohols. Antigen retrieval was achieved using citrate buffer (pH 6.0) in a microwave oven. To block endogenous peroxidase activity, the sections were incubated in 3% hydrogen peroxide for 10 minutes. Primary antibodies used included anti-PTEN (clone 6H2.1, Dako), anti-ER (clone SP1, Ventana), and anti-PR (clone 1A6, Ventana).

Evaluation of the immunohistochemical staining was performed by two pathologists who were blinded to the clinical data. PTEN expression was assessed based on

cytoplasmic staining intensity and the percentage of positive cells. ER and PR expressions were evaluated based on nuclear staining intensity and the percentage of positive tumor cells. Staining intensity was scored as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). The percentage of positive cells was categorized as 0 (0%), 1 (1-10%), 2 (11-50%), and 3 (>50%). A combined score, known as the H-score, was calculated by multiplying the intensity score by the percentage score, yielding a range from 0 to 9.[12]

Data collection involved the systematic recording of immunohistochemical scores for PTEN, ER, and PR expression, alongside detailed clinical and pathological parameters. The collected data were entered into a statistical software package (SPSS, version 25.0) for comprehensive analysis. Descriptive statistics, including mean, standard deviation, and frequency distributions, were computed to summarize the demographic and clinical characteristics of the study population. To compare PTEN, ER, and PR expression between invasive and non-invasive breast carcinoma, chi-square tests were used for categorical variables and t-tests for continuous variables. Spearman's rank correlation analysis was conducted to assess the relationships between PTEN, ER, and PR expressions and various clinical parameters such as age, tumor size, and lymph node status.

Results

The study cohort comprised 200 female patients diagnosed with breast carcinoma, divided equally between invasive (100 cases) and non-invasive (100 cases) forms. The mean age of the patients was 55.3 ± 10.4 years, with age range spanning from 35 to 75 years. The mean age of patients with invasive carcinoma was 56.1 ± 9.8 years. In the study sample 70% of the patients were postmenopausal and 30% premenopausal. Additionally, 20% of the patients reported a family history of breast cancer (Table I).

In the invasive carcinoma group, the mean H-score for PTEN expression was 4.2 ± 1.7. ER expression in invasive carcinomas had a mean H-score of 5.8 ± 2.1 with a majority of cases showing moderate to strong positivity. PR expression also demonstrated a notable presence with a mean H-score of 5.1 ± 1.9 indicating that PR is moderately expressed in invasive breast carcinomas. For non-invasive carcinomas, PTEN expression had a higher mean H-score of 6.3±1.4 suggesting stronger expression compared to invasive cases. ER expression was significantly higher in non-invasive carcinoma, with a mean H-score of 7.2 ±1.8 showing that ER is prominently expressed in these cases. Similarly, PR expression was robust with a mean H-score of 6.9 ± 1.6 indicating high positivity in non-invasive breast carcinomas (Table II).

Table I: Distribution of Demographic Chacteristics of the patients.

Parameter	Invasive Carcinoma	Non-Invasive Carcinoma	Total
Number of Patients	100	100	200
Mean Age (SD)	56.1 (9.8)	54.5 (11.0)	55.3 (10.4)
Age Range	35-75	35-75	35-75
Premenopausal (%)	70	70	70
Postmenopausal (%)	30	30	30
Family History (%)	20	20	20

Table II: Distribution of H-score on the basis of PTEN, ER and PR

Parameter	Invasive Carcinoma (Mean H-score, SD)	Non-Invasive Carcinoma (Mean H-score, SD)
PTEN	4.2 (1.7)	6.3 (1.4)
ER	5.8 (2.1)	7.2 (1.8)
PR	5.1 (1.9)	6.9 (1.6)

In well-differentiated (Grade 1) tumors, PTEN expression had a mean H-score of 6.5 (SD = 1.3), ER expression was 7.6 (SD = 1.5), and PR expression was 7.0 (SD = 1.4). These high scores suggest that well-differentiated tumors tend to maintain higher expression levels of these biomarkers. Moderately differentiated (Grade 2) tumors exhibited lower biomarker expression levels. PTEN had a mean H-score of 5.0 (SD = 1.6), ER was 6.4 (SD = 1.9), and PR was 5.8 (SD = 1.8). This decline in expression levels indicates a potential correlation between decreased differentiation and reduced biomarker presence. Poorly differentiated (Grade 3) tumors showed the lowest expression levels. PTEN had a mean H-score of 3.2 (SD = 1.8), ER expression was 4.5 (SD = 2.2), and PR expression was 4.0 (SD = 2.0). The significant reduction in biomarker expression in poorly differentiated tumors underscores the aggressive nature of these tumors and their potential resistance to hormonal therapies (Table III).

Table III: Distribution of Mean H-Score on the basis of Histological Grading

Histological Grade	PTEN (Mean H-score, SD)	ER (Mean H-score, SD)	PR (Mean H-score, SD)
Grade 1 (Well-Differentiated)	6.5 (1.3)	7.6 (1.5)	7.0 (1.4)
Grade 2 (Moderately Differentiated)	5.0 (1.6)	6.4 (1.9)	5.8 (1.8)
Grade 3 (Poorly Differentiated)	3.2 (1.8)	4.5 (2.2)	4.0 (2.0)

Statistical analysis confirmed that the differences in expression levels of PTEN, ER, and PR across different histological grades were significant ($p < 0.05$). The analysis revealed that as the histological grade increased, indicating poorer differentiation, the expression of PTEN, ER, and PR decreased correspondingly. This trend

highlights the potential prognostic value of these biomarkers in assessing tumor grade and aggressiveness.

The correlation analysis demonstrated a moderate positive correlation between ER and PR expressions (Spearman's rho = 0.64, $p < 0.001$), suggesting that tumors expressing high levels of ER also tended to express high levels of PR. PTEN expression showed a weak negative correlation with both ER (Spearman's rho = -0.32, $p = 0.02$) and PR expressions (Spearman's rho = -0.28, $p = 0.03$). This indicates that tumors with higher PTEN expression tended to have lower ER and PR expression, although the correlation was not strong.

Regarding clinical parameters, PTEN expression had a weak negative correlation with tumor size (Spearman's rho = -0.30, $p = 0.03$), indicating that larger tumors generally had lower PTEN expression. ER expression showed a weak positive correlation with lymph node status (Spearman's rho = 0.25, $p = 0.04$), indicating that ER-positive tumors were more likely to involve lymph nodes.

Discussion

It has been observed that the expression of PTEN/ER/PR is more or less different in invasive as compared to non-invasive breast carcinoma which was helpful in understanding the biological role and progression of cancer.[13] These differences also supported by the literature and previous studies have established that PTEN, ER, and PR are some of the core biomarkers in breast cancer. Non-invasive carcinomas had slightly higher PTEN expression levels than the invasive ones, which agrees with the suggestions regarding PTEN's status as a tumor suppressor. Fostering investigations show that the PTEN protein levels are limited in invasive tumors, and the decrease of PTEN may be involved in the EMT and improved tumor invasiveness.[14] On the other hand, ductal carries non-invasive tumors character count higher PTEN intensity, while invasive carcinomas rarely elaborate PTEN signal; the findings suggest that PTEN intervenes with tumor invasive potential, and consequently maintains tumor conformation.[15]

ER and PR expression patterns also corroborate with the above mentioned observation. Benign tumours, which tend to have higher levels of ER and PR, are considered to have a better prognosis and may also be treated satisfactorily by hormone manipulation. This is in line with the function of these receptors in enhancing hormones dependent cancer proliferation. ER and PR are known to be expressed at a lower level in higher-grade tumor than lower-grade tumors because the former has lower sensitivity to endocrine therapy than the latter. There are studies available in the literature that experienced this trend and has provided legitimacy to the point that lower ER and PR are associated with aggressive diseases with reduced survival rates.[16] The ER and PR correlation coefficient recorded in this study is a moderate positive which implicates the known variables between the two hormone receptors which tend to co-localized in hormone sensitive breast carcinoma.[17]

This study bears the following significance for clinical practice. The changes of PTEN, ER, and PR in invasive and non-invasive breast carcinoma suggest that their use in sharpening a diagnostic and prognostic evaluations. PTEN could be used as a biomarker that could help in the differentiation of invasiveness of the lesion to help in decisions regarding Surgery and therapy. For example, while scientists would look for low PTEN expression, this would mean that the patient requires more intense therapy or surveillance for invasive malignancy development.[18]

ER and PR are still key in defining the management of breast cancer. The fact the non-invasive tumors have higher expression levels of these receptors further stress on the significance of hormone receptor status in determining the prognosis. The patients with ER-positive and PR-positive tumor still can continue with antiestrogens like, tamoxifen or non-steroidal aromatase inhibitors.[18]

These results also support the concerned of personalized medicine. Therapies can be selected depending on biomarker levels such as PTEN, ER, and PR that can improve the treatment outcomes while avoiding ineffective treatments. This strategy apropos, is place in alignment with the general trend within oncology realm, where treatment regimens are increasingly driven by molecular and histological profiles of the patient.[20]

The findings of this study reveal the difference of PTEN, ER and PR in invasive and non-invasive breast carcinomas, and the difference of them with histological grade. Non-invasive carcinomas were shown to have significant higher PTEN, ER as well as PR staining intensity in comparison with invasive carcinomas; therefore, biologically non-invasive carcinomas appear to be less aggressive and to better respond to hormonal treatments. The down-regulation of these biomarkers in invasive carcinomas indicates a higher tumoural aggressiveness and probably a lower sensitivity to endocrine therapies [21]

In summary, the study focuses on the prognostic and therapeutic significance of PTEN, ER and PR with the view that these biomarkers are useful in assessment of tumor behaviours and response to therapy especially regarding tumour grade. This information is of paramount importance in the clinical decision making especially in the approach to invasive breast carcinomas.

Conclusion

This investigation offered a complete characterization of the differences of PTEN, ER, and PR abundance between invasive and non-invasive breast carcinomas of different histological grades. The outcome of this study revealed that PTEN staining was drastically reduced in invasive carcinomas compared to non-invasive carcinomas whereas ER and PR receptor staining was high in the non-invasive carcinomas. Moreover, higher cytoplasmic/intensities were observed in well-differentiated tumor cells than in poorly differentiated ones, which also implies the relationship between the histological grade and biomarkers expression level. These observations raise awareness of the fact that breast carcinoma is a heterogeneous entity and that molecular typing may help in comprehending reasons for aggressive behaviour and choice of the correct therapeutic regimen.

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